Original Investigation

Value of ^{18}Fluorodeoxyglucose–Positron-Emission Tomography in Amyotrophic Lateral Sclerosis
A Prospective Study

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IMPORTANCE Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disorder primarily affecting the motor system, with extramotor involvement to a variable extent. Biomarkers for early differential diagnosis and prognosis are needed. An autosomal dominant hexanucleotide (GGGGCC) expansion in the noncoding region of the chromosome 9 open reading frame 72 (*C9orf72*) gene is the most frequent genetic cause of ALS, but its metabolic pattern has not been studied systematically.

OBJECTIVES To evaluate the use of ^{18}fluorodeoxyglucose–positron-emission tomography as a marker of ALS pathology and investigate whether a specific metabolic signature is present in patients with *C9orf72* mutations.

DESIGN, SETTING, AND PARTICIPANTS In total, 81 patients with a suspected diagnosis of ALS at University Hospital Leuven were prospectively investigated. All underwent detailed neurological examination and electrodiagnostic and genetic testing for the major known genetic causes of ALS (*C9orf72*, *SOD1*, *TARDBP*, and *FUS*). A diagnosis of ALS was made in 70 of 81 patients. Of these, 11 were *C9orf72* positive and 59 were *C9orf72* negative. In 7 patients, the diagnosis of primary lateral sclerosis was made; 4 patients had progressive muscular atrophy. A screened healthy control population was used for comparison.

MAIN OUTCOMES AND MEASURES Positron-emission tomographic data were spatially normalized and analyzed using a predefined volume of interest and a voxel-based analysis (SPM8). Discriminant analysis was done both volume of interest based and voxel based using a support vector machine approach.

RESULTS Compared with control participants, ^{18}fluorodeoxyglucose–positron-emission tomography showed perirolandic and variable prefrontal hypometabolism in most patients. Patients with primary lateral sclerosis showed a similar pattern. Patients with *C9orf72*-positive ALS had discrete relative hypometabolism in the thalamus and posterior cingulate compared with those with *C9orf72*-negative ALS. A posteriori-corrected discriminant analysis was able to correctly classify 95% of ALS cases and 71% of primary lateral sclerosis cases. Prefrontal hypometabolism was associated with reduced clinical functioning (ALS Functional Rating Scale). Extensive hypometabolism in the prefrontal or anterior temporal areas was present in 10% of patients and associated with significantly shorter survival as an independent factor (n = 63, $P < .001$). Patients who were *C9orf72* positive did not differ in survival compared with those who were *C9orf72* negative.

CONCLUSIONS AND RELEVANCE ^{18}Fluorodeoxyglucose–positron-emission tomography is a useful early diagnostic and prognostic marker for ALS. Amyotrophic lateral sclerosis that is positive for *C9orf72* is characterized by only mild cerebral metabolic differences that show no prognostic difference.

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Motor neuron disorders (MNDs) comprise a group of disorders involving preferential damage to upper motor neurons (UMNs) and/or lower motor neurons (LMNs).[1,2] In adulthood, the clinical spectrum of MNDs is wide, ranging from simultaneous involvement of UMNs and LMNs (classic amyotrophic lateral sclerosis [ALS], about 90% of all MND cases), a pure UMN syndrome (primary lateral sclerosis [PLS]), and an isolated LMN involvement (progressive muscular atrophy [PMA]).[1] It has not been settled whether the latter 2 are entities fully separated from ALS because a considerable number of patients with PLS and PMA progress to ALS.

A diagnosis of ALS predominantly relies on the interpretation of clinical symptoms and signs, with the use of ancillary tests to exclude other causes.[1-3] Currently, the revised El Escorial and Awaji-Shima diagnostic criteria are used, the latter being more sensitive for early disease assessment.[4] Upper motor neuron symptoms are spasticity, hyperreflexia, and the Babinski sign, whereas LMN loss leads to muscle weakness and wasting with fasciculations. Depending on symptom onset, clinical presentations of ALS may be separated into bulbar or spinal-onset disease.

It is now recognized that ALS and PLS are characterized by significant extramotor cerebral pathology that, to a variable extent, overlaps with the clinicopathological features of frontotemporal lobar degeneration (FTLD),[5] and cognitive decline may range from mild abnormalities to manifest FTLD in about 5% to 15% of cases.[6]

Although most MND patients are sporadic, approximately 10% have a positive family history.[6] Previously, the most prevalent and best-known mutation was in the Cu/Zn superoxide dismutase 1 gene (*SOD1*), whereas other genes that cause autosomal-dominant ALS (such as *TARDBP*, *FUS*, *VAPB*, *ANG*, *OPTN*, *UBQLN2*, and *ATXN2*) only accounted for a small percentage of cases. In 2011, a new gene defect was identified, namely, a massively expanded GGGGCC hexanucleotide repeat in a noncoding region of the chromosome 9 open reading frame 72 (*C9orf72*) gene.[7,8] Phenotypically, patients with the expansion have a younger age at onset, shortened survival, increased rates of cognitive and behavioral impairment, and a strong family history of neurodegenerative disease.[9-16] This mutation is currently recognized as a major genetic cause of ALS and FTLD, responsible for up to 40% of familial ALS, 5% to 10% of sporadic ALS, about 20% of familial FTLD, and 5% of sporadic FTLD.[15,17-20]

At the neuropathological level, most forms of ALS are characterized by the presence of inclusions in degenerating motor neurons staining positively for ubiquitin and TAR DNA-binding protein 43. Whereas the presence of LMN degeneration can be confirmed by electrodiagnostic testing, there is no widely accepted biomarker for UMN involvement.[21] However, reliable objective biomarkers are critical for the early diagnosis, monitoring of disease progression, prognosis, and patient stratification for clinical trials.

Whereas magnetic resonance imaging (MRI) of the brain and spinal cord remains a most useful neuroimaging technique in ALS, this is mainly to exclude syndromes that mimic ALS.[6] Voxel-based morphometry reveals regional atrophy, and magnetic resonance spectroscopy and diffusion-tensor MRI can detect corticospinal lesions. However, because of their relative lack of sensitivity and specificity, these techniques are currently inadequate for use as diagnostic tools in individual patients.[1,22,23]

Early ^{18}fluorodeoxyglucose (FDG)-positron-emission tomography (PET) imaging studies conducted in limited numbers of patients (n < 20) and dating back 25 years have shown that patients with ALS have decreased glucose uptake that is more extended than motor areas.[24-27] Frontal hypometabolism has been associated with neuropsychological deficits,[27,28] especially to disturbances of word fluency.[26,29] Detecting extramotor involvement is important because comorbid cognitive dysfunction has been associated with functional decline, shortened survival, and poor compliance with life-prolonging interventions.[30,31]

Voxel-based approaches have led to a more detailed, group-based characterization of the disorder spectrum. In a study in 32 patients, the pattern of UMN (bulbar) and LMN (spinal) onset could be differentiated by FDG-PET.[32] To our knowledge, the individual discrimination capacity of FDG-PET as an aid in early and differential diagnosis in MND has not been studied.

The aim of this study was 3-fold: first, to evaluate glucose metabolism in a large group of patients with ALS and those with PLS, in comparison to age-matched healthy control participants, to assess the diagnostic value of voxel-based classification. Second, our goal was to study the metabolic impact of the *C9orf72* mutation in patients with ALS. And third, we aimed to relate survival of patients with ALS associated with the presence of frontotemporal hypometabolism.

Methods

Patients (n = 81) were consecutively recruited from referrals made to the neuromuscular clinic at the University Hospital Leuven (Leuven, Belgium) between January 2011 and January 2013. None of the patients had a history of other neurological disorders. All underwent full neurological evaluation and electrodiagnostic testing as part of their clinical workup by an experienced specialist in neuromuscular disorders (P.V.D. or W.R.). Both the revised El Escorial and Awaji-Shima criteria were applied.[4] All patients underwent FDG-PET planned at the initial visit. All patients had undergone a routine MRI scan of the brain. All patients underwent genetic testing for *C9orf72*, *SOD1*, *TARDBP*, and *FUS* at the time of diagnosis. A *C9orf72* repeat expansion was checked for by repeat-primed polymerase chain reaction, and mutations in *SOD1*, *TARDBP*, and *FUS* were as sought for by Sanger sequencing, as previously described.[15,33-35]

Onset of the disease was determined by the patient's recollection of the month in which the first symptoms occurred. None of the patients showed evidence of respiratory compromise or nutritional abnormalities, such as dehydration or ketosis, at the time of the FDG-PET scan. The functional status of the patients near the time of PET imaging was scored by the revised version of the ALS Functional Rating Scale (FRS) in most patients. Also, forced vital capacity as a marker of lung func-

Table 1. Demographic and Clinical Characteristics of the Study Participants

Characteristic	Mean (SD)					
		ALS				
	Control	All	*C9orf72* Negative	*C9orf72* Positive	PLS	PMA
No.	20	70	59	11	7	4
Sex, No.						
Male	12	44	37	7	2	3
Female	8	26	22	4	5	1
Age at onset, y		60.4 (12.6)	60.8 (13.1)	58.5 (9.4)	52.4 (13.1)	66.2 (5.3)
Diagnostic delay, mo		12.6 (10.1)	12.7 (10.3)	12.5 (9.8)	47.1 (52.7)[a]	12.5 (5.3)
Age at PET, y	62.4 (6.4)	62.1 (12.5)	62.6 (13.0)	61.2 (9.2)	57.5 (12.9)	67.5 (5.6)
Time onset to PET, mo		15.2 (10.7)	15.0 (10.6)	13.8 (10.2)	52.3 (52.1)[a]	12.5 (5.7)
Time diagnosis to PET, mo		2.1 (3.5)	2.3 (3.8)	1.3 (1.0)	5.3 (15.1)	0.1 (0.6)
Awaji-Shima diagnostic category, definite/probable/possible, No.		32/29/9	21/29/9	11/0/0		
Onset type, S/B		48/21	40/18	8/3	6/1	3/1
FVC, %		93.9 (24.0)	93.3 (21.6)	96.8 (35.0)	119.0 (9.3)[a]	97.7 (35.0)
ALS FRS-R score		36.3 (7.2) [n = 46]	36.2 (6.1)[n = 40]	32.5 (12.7) [n = 6]	39.2 (6.9)[n = 4]	41.2 (3.4)[n = 4]

Abbreviations: ALS, amyotrophic lateral sclerosis; B, bulbar; FRS-R, Functional Rating Scale–Revised; FVC, forced vital capacity; PET, positron-emission tomography; PLS, primary lateral sclerosis; PMA, progressive muscular atrophy; S, spinal.

[a] $P < .05$ compared with all ALS.

tion was scored (**Table 1**). Control participants were part of a prospective study in healthy volunteers aged 50 to 80 years acquired over the same period on the same equipment. Inclusion and exclusion details for this substudy are given in eAppendix 1 in Supplement.

The study was approved by the local university ethics committee and written informed consent was given by patients and control participants, according to the Declaration of Helsinki. Positron-emission tomography acquisition, processing, and statistics are provided in eAppendix 1 in Supplement. Patient characteristics are given in eAppendix 2 in Supplement and Table 1.

Results

Categorical Differences of ALS vs Control Cases

On an individual visual level, as diagnosed in clinical routine using surface projections and z-maps, FDG-PET showed areas of hypometabolism in virtually all individual patients with ALS and those with PLS, with variable intensity ranging from very mild to severe. The most common regions with hypometabolism clinically reported were the perirolandic and frontal brain regions.

Voxel-based group analysis showed that, compared with healthy control participants, patients with ALS had significant, symmetrical hypometabolism in the prefrontal, lateral frontal, and premotor cortex. Patients with ALS showed also clusters of relative hypermetabolism in the cerebellum, occipital cortex, upper brain stem, and medial temporal cortex, encompassing the hippocampus and amygdala (**Figure 1**A and eTable 1 in Supplement for cluster statistics and locations). In 3 of 4 patients diagnosed as having PMA, a similar pattern of hypometabolism was seen. One patient with PMA, with a mutation in *SOD1*, had a visually normal FDG-PET.

Categorical Differences of *C9orf72* Negative vs *C9orf72* Positive

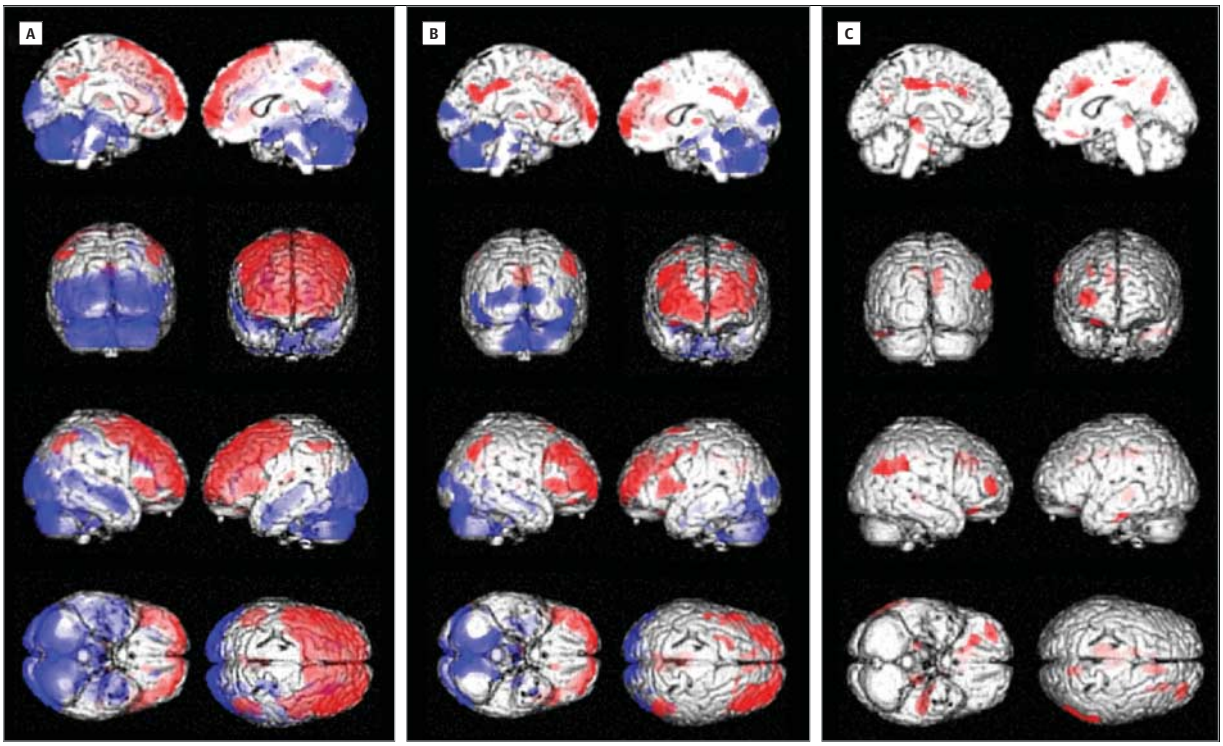
There were no significant differences in patients with *C9orf72*-positive vs *C9orf72*-negative ALS in time of disease onset to PET, bulbar vs spinal onset, forced vital capacity measurements, or ALS-FRS values. Patients with *C9orf72*-positive ALS showed a similar pattern of hypometabolism compared with control participants, slightly more pronounced bilaterally in the thalamus and posterior cingulate and precuneus (Figure 1B and eTable 1 in Supplement). These results were confirmed by volume of interest (VOI)-based analysis of variance, with a difference for the posterior cingulate of 4.0% ($P = .003$, post hoc–corrected univariate analysis of variance) (eFigure 1 in Supplement).

Direct comparison of *C9orf72*-positive ALS vs *C9orf72*-negative ALS showed no differences at the preset thresholds. Exploratively, at a less stringent threshold of significance, patients with *C9orf72*-positive ALS showed relatively lower metabolism in the anterior and posterior cingulate, posterior thalami, right lateral frontal cortex, and right temporoparietal junction (Figure 1C and eTable 1 in Supplement).

Categorical Differences of ALS vs PLS

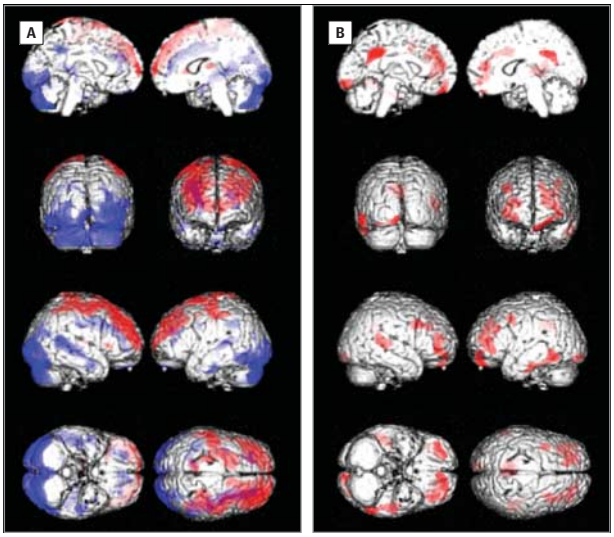
Patients with PLS also showed symmetrically decreased metabolism bilaterally in the prefrontal cortex, anterior cingulate, pericentral cortex, and thalamus (**Figure 2**A and eTable 2 in Supplement). Relative hypermetabolism was also present in the cerebellum, occipital cortex, and left lateral temporal cortex.

Figure 1. Relative Glucose Metabolism in *C9orf72*-Positive and *C9orf72*-Negative Patients With Amyotrophic Lateral Sclerosis (ALS) and Control Cases



A, Surface and interhemispheric projections of areas with relative hypometabolism (red) and hypermetabolism (blue) for patients with ALS vs healthy control cases (P_{height} < .001). B, Patients with *C9orf72*-positive ALS vs healthy control cases (P_{height} < .001). C, Patients with *C9orf72*-positive vs those with *C9orf72*-negative ALS (P_{height} < .005).

Figure 2. Relative Glucose Metabolism in Primary Lateral Sclerosis (PLS) vs Controls and vs Amyotrophic Lateral Sclerosis (ALS)



A, Surface and interhemispheric projections of areas with relative hypometabolism (red) and hypermetabolism (blue) for patients with PLS vs healthy control cases (P_{height} < .001). B, Patients with PLS vs those with ALS (P_{height} < .005).

Direct comparison between PLS and ALS on a group level showed more severe metabolic involvement of the prefrontal cortex and posterior cingulate in ALS (P_{height} < .005; k_E = 200; Figure 2B and eTable 1 in Supplement for cluster locations and statistics). The inverse contrast showed lower metabolism in the primary sensorimotor cortex in PLS in bilateral clusters, which was only significant after small-volume correction (P_{FDR} < .05; T statistic, 3.99 left and 3.76 right) based on the a priori spatial restriction of known primary motor cortex involvement in MND (data not shown in Figure 2B).

Discriminant Analysis

VOI Based

To evaluate the use of FDG-PET to discriminate between different forms of motor neuron degeneration, a discriminant analysis was performed. In total, 88 VOI regions were used for discriminant analysis between control participants, patients with ALS, and patients with PLS. Using equal a priori probabilities as the most conservative estimation, the classification matrix after leave-one-out cross-validation (**Table 2**) shows an overall classification accuracy of 89.7% (91.8% without cross-validation). The most discriminating regions were the prefrontal cortex, thalamus, posterior cingulate, and anterior cingulate (**Figure 3**). In total, 10 of 97 individuals were misclassified: 4 ALS cases as control participants, 4 ALS cases as PLS cases, and 2 PLS cases as ALS cases. eFigure 2 in Supplement shows the canonical discriminant function values with classification plot.

When including patients with PMA in the analysis, cross-validated classification accuracy dropped to 62.4%, as the cen-

Table 2. Stepwise Forward Discriminant Analysis[a]

Group	% Correct	Control	ALS	PLS
Control	100.0	20	0	0
ALS	88.6	4	64	3
PLS	71.4	0	2	5
Total	89.7	24	66	8

Abbreviations: ALS, amyotrophic lateral sclerosis; PLS, primary lateral sclerosis.

[a] Values with equal a priori probabilities for separation between control cases, patients with ALS, and patients with PLS after leave-one-out cross-validation.

troid of the PMA group was very close to ALS, and discriminative features of motor cortex and thalamic dysfunction were not noted for PMA (Figure 3). However, because of the very small PMA group, these findings have to be interpreted with caution.

In the clinically relevant setting of only patients with ALS vs control participants (ie, when a priori clinical information was included), overall classification accuracy was 94.4% with 5 ALS cases misclassified as control cases. Discriminant analysis could not separate *C9orf72*-negative and *C9orf72*-positive ALS with an accuracy greater than 60%, confirming the high overlap between both groups of patients.

Support Vector Machine Analysis

To further refine the discriminating regions in patients with ALS and those with PLS, a support vector machine (SVM) analysis was performed. The classifier image between ALS and control participants is shown in **Figure 4**A, and the individual SVM distances are given in Figure 4B. The most important clusters of discrimination by SVM were found bilateral in the thalamus, primary motor cortex, striatum, prefrontal and lateral prefrontal cortex, and posterior cingulate. For ALS vs control cases, the leave-one-out approach had a sensitivity of 94.8, a specificity of 80.0%, and an accuracy of 91.8%. Similarly, for the subgroups of patients with *C9orf72*-negative ALS, sensitivity, specificity, and accuracy were 89.8%, 85.0%, and 88.6%, respectively. For patients with *C9orf72*-positive ALS, these were 90.9%, 100%, and 96.8%, respectively.

For PLS vs control cases, these values decreased to 57.1% for sensitivity (specificity 100%). An SVM was not able to discriminate between *C9orf72*-positive and *C9orf72*-negative ALS (sensitivity 0%), in concordance with the small differences found by voxel-based group comparison.

Survival vs Frontotemporal Metabolism

At the time of the writing of this article, 20 of 70 (28.6%) of this patient cohort had died (mean [SD] survival after symptom onset in these patients, 19.9 [7.1] months). For all surviving patients (50 of 70), more than 14 months of follow-up after disease onset was available (average [SD], 39.9 [16.9] months) and 48 of 50 (96%) of the surviving patients were beyond the point of the 20-month disease duration.

In 7 of 70 patients (10%), large areas with a more than 2 SD decreased uptake in frontal and/or anterior temporal regions were observed. Of these, 6 of 7 patients had died after surviving a mean (SD) of only 20.0 (7.0) months (**Figure 5**A; for clinical details of these patients, see eTable 3 in Supplement). The lower survival in the patients with ALS with severe frontotemporal metabolic involvement was highly significant ($P < .001$). This shortened survival was not owing to the contribution of *C9orf72*-positive patients (Figure 5B, $P = .45$). In this cohort, no correlation between survival and site of onset ($P = .46$) was found. After correction for other prognostic factors, such as age at onset ($P = .001$; hazard ratio, 1.08; 95% CI, 1.03-1.13) and forced vital capacity ($P = .01$; hazard ratio, 0.98; 95% CI, 0.96-0.99), the extensive hypometabolism on FDG-PET remained significant ($P = .005$; HR, 4.1; 95% CI, 1.6-11.0).

The correlation between frontal hypometabolism and ALS FRS is described in eAppendix 2 and eFigure 3 in Supplement.

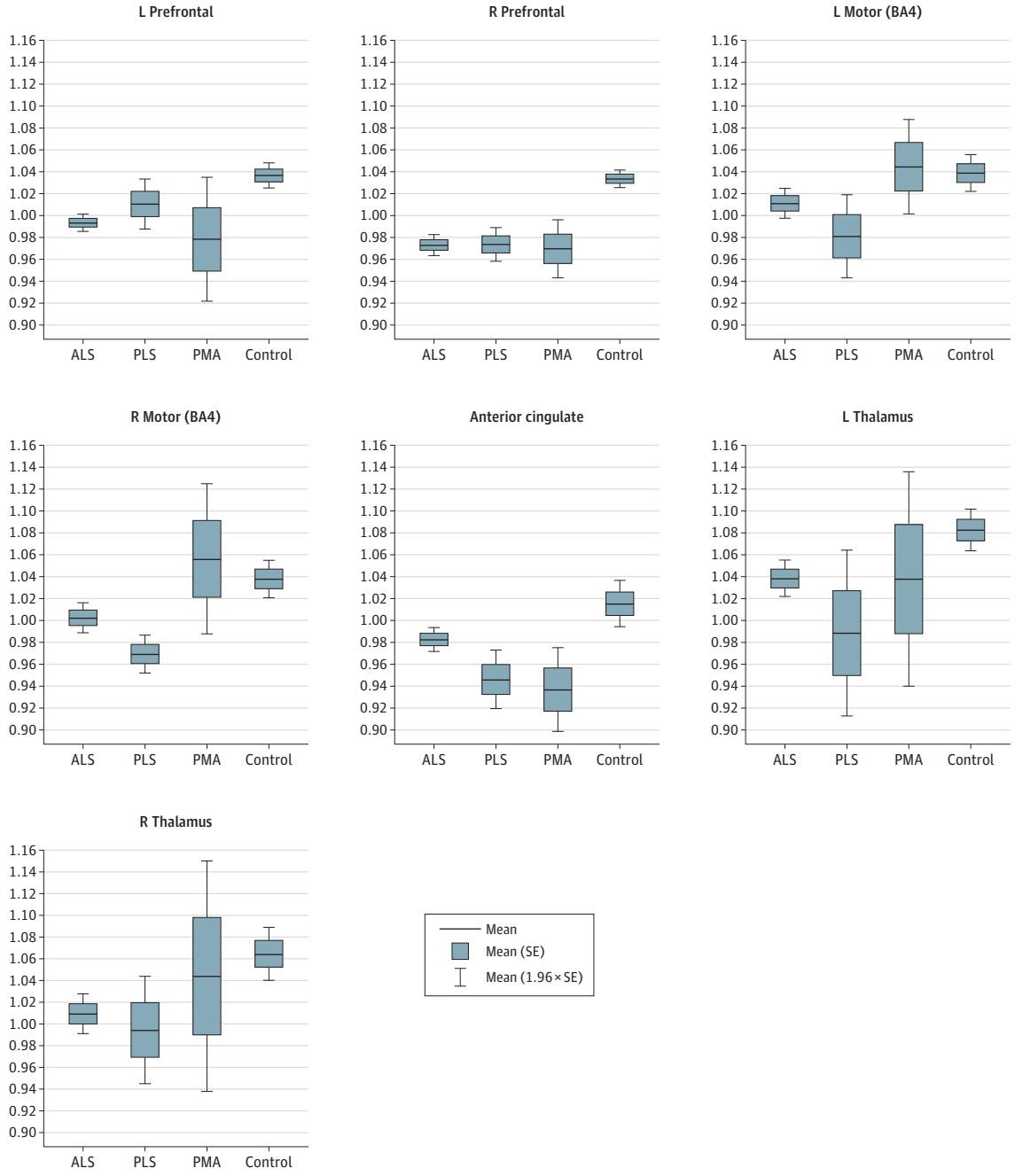
Discussion

Reliable diagnostic and disease-state biomarkers of motor neuron degeneration would facilitate the management and clinical trials of patients with ALS.[23] Imaging techniques, especially MRI sequences, have shown their potential to track cerebral motor and extramotor pathology in ALS.[36,37] Conventional MRI findings—such as corticospinal tract hyperintensities on fluid-attenuated inversion recovery, T2-weighted, and proton density images or a hypointense rim at the margin of the precentral gyrus in T2-weighted images—are frequently found in patients with ALS, but the overlap with control cases is high and therefore lacking specificity.[38] Voxel-based morphometry and cortical thickness measurements have revealed atrophy in the motor cortex and frontal involvement in patients with ALS. Diffusion-tensor imaging of the corticospinal tract shows a significantly decreased fractional anisotropy in patients with ALS and UMN degeneration but does not provide sufficient discriminatory power at the individual level. Magnetic resonance spectroscopy of the motor cortex has shown abnormally low *N*-acetylaspartate levels in patients with advanced ALS that is correlated with verbal fluency deterioration,[39] but studies at the early stage of the disease and with an adequate follow-up are scarce.[40] Also, previous FDG-PET studies in smaller sample sizes have shown significant group differences but have not focused yet on classification accuracy on the level of an individual patient.[24-27]

Advanced classification techniques using whole-brain analytic approaches have the potential to ameliorate the accuracy of discrimination on an individual patient level. Therefore, in this study, we evaluated the value of FDG-PET in a large prospective cohort of patients with ALS around the time of diagnosis using whole-brain region-based discriminant and SVM tools.

To our knowledge, this study is the first to report on glucose metabolic patterns on a group basis in a cohort of patients with *C9orf72*-positive ALS. So far, only cases of *C9orf72* ALS with FDG-PET and 99mTc-ECD perfusion single-photon emission computed tomography have been described, each in

Figure 3. Most Discriminative Areas of Reduced Glucose Metabolism in Amyotrophic Lateral Sclerosis (ALS) and Primary Lateral Sclerosis (PLS)



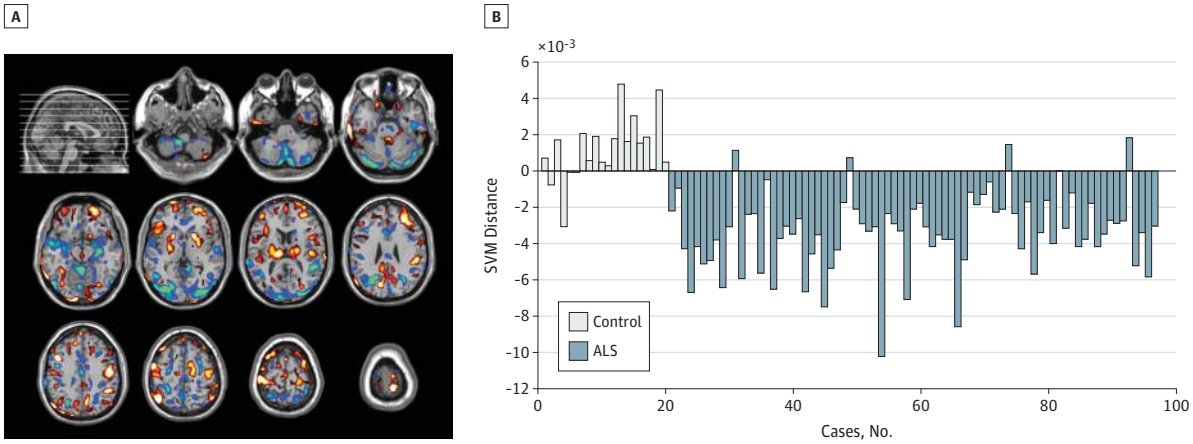
Box-and-whisker plots of relative glucose metabolism (normalized to mean gray matter value) in volume of interest areas that are most discriminative between healthy control cases, patients with ALS, and patients with PLS: prefrontal (Brodmann Area [BA]9), motor cortex (BA4), anterior cingulate (BA 24,32), and thalamus. L indicates left; PMA, progressive muscular atrophy; R, right.

5 patients with combined ALS-frontotemporal dementia.[19] Concordant with this study, large heterogeneity in individual metabolic patterns is found. The frequency of the *C9orf72* mutation in our cohort was very similar to that previously reported series of patients with ALS (20%-50% of familial ALS and 4%-10% of sporadic ALS).[20,41]

We found that perirolandic and prefrontal hypometabolism is a sensitive marker of ALS. *C9orf72*-positive patients had a similar pattern, but the involvement of the posterior cingulate, thalamus, and prefrontal cortex was on average slightly more severe. Similar affected areas were observed in a study with diffusion-tensor MRI.[42]

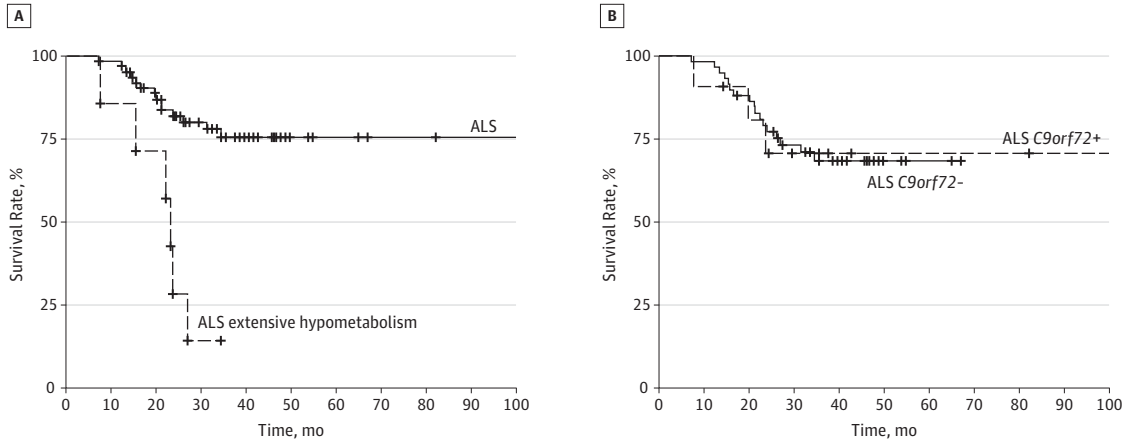
Our findings are consistent with previous PET studies in smaller samples, where metabolic abnormalities in the dorsolateral prefrontal cortex and anteromedial cingulate cortex were the most consistent findings. In *C9orf72*-negative ALS,

Figure 4. Support Vector Machine (SVM) Voxel-Based Discriminant Analysis of Amyotrophic Lateral Sclerosis (ALS) vs Control Cases



A, Feature weights of the classifier for ALS vs healthy control cases projected onto a normalized structural magnetic resonance image in Montreal Neurological Institute space. The scale of the feature weights represents how much a voxel contributes. The scale was normalized so that the sum of all weights is 1. Only voxels with a weight of more than 0.002 in absolute value are shown. Clusters indicate areas with high discriminative impact based on relative hypometabolism (yellow-red) and relative hypermetabolism (blue). B, Plots of distance to the classifier for healthy control cases (green) vs patients with ALS (*C9orf72* positive and *C9orf72* negative grouped together) using a leave-one-out approach.

Figure 5. Survival Plots of Patients With Amyotrophic Lateral Sclerosis (ALS) With and Without Extensive Frontotemporal Hypometabolism



Extensive hypometabolism in frontotemporal areas is a negative prognostic factor. A, Kaplan-Meier survival plots of patients with ALS with (n = 7) vs without (n = 63) extensive frontotemporal hypometabolism ($P < .001$). B, Kaplan-Meier survival plots of patients with ALS with (*C9orf72*+, n = 11) or without (*C9orf72*−, n = 59) the *C9orf72* repeat expansion ($P = .45$).

extramotor involvement has been studied before and consistently reported,[24,25] although not all studies found resting-state hypometabolism or hypoperfusion.[43]

As was previously reported by Cistaro et al,[32] relative hypermetabolism in the posterior areas, including the cerebellum, occipital cortex, brain stem, and mesial temporal, is also seen. Although this can purely be due to the relative normalization procedure used, it has been suggested that this pattern could reflect true hypermetabolism and a measure of astrocytosis or microglial activation in these areas. This question of true hypermetabolism should be addressed by absolute regional cerebral metabolic rates of glucose measurements to enable definite conclusions. In the same study, hypermetabolism in the upper brain stem was particularly described in spinal-onset forms only, but this was not confirmed in our study, where both patients with spinal and bulbar onset ALS showed completely similar patterns of hypometabolism (data not shown).

The correlation between the ALS-FRS scores and prefrontal hypometabolism is congruent with previous reports showing that executive impairment is related to overall functioning of patients. Lower ALS-FRS scores are also associated with poorer prognosis.[6] Extensive hypometabolism in the frontal or temporal lobes was associated with shorter survival after correction for other known prognostic factors. This is in line with other studies showing a negative correlation between con-

comitant frontotemporal dementia or cognitive or behavioral impairment and survival.[30,31]

From a clinical point of view, this study has shown that, although deviations from normality are relatively modest and some variability exists, the pattern of affection in patients with ALS and those with PLS does allow for an accurate discrimination with healthy control cases, on a quantitative basis, and even between patients with PLS and ALS, albeit with lower accuracy and only tested in a limited group of patients with PLS. The discrimination of patients with ALS using FDG-PET could be an important additional tool in early diagnoses of new cases and in the assessment of disease prognosis. And both the VOI- and SVM-based analyses performed in this group remain to be validated in a novel prospective cohort of patients.

Our findings also support the notion that PLS is part of the wider MND spectrum. The corollary is that the modifying factors (genetic and environmental) that account for the delayed progression of disease in patients with PLS, and which are not clearly reflected by major metabolic differences as resulting from this study, remain to be discovered.

A number of limitations of the study need to be mentioned. First, although routine MRI was performed in all patients, we did not apply a volume correction on the FDG-PET of the participants. Atrophy has been described in patients with ALS,[44-47] but as for most patients, no volumetric MRI data were available; no partial volume correction was possible in this dataset. This does not diminish the validity of the results and their indication of discriminatory power in the functional metabolic information, which is a measure of implicit cellular metabolism and macroscopic atrophy effects.

Second, we did not assess the issue of laterality, which may have caused some levelling out of information in possible larger deviations. In most patients, however, relatively symmetric cerebral cortical involvement seems to be the rule, and in previous studies, left-right (metabolic or perfusion) asymmetry has not been regarded as a feature of ALS. Third, we did not perform extensive cognitive testing in our patients. Therefore, we cannot correlate the FDG-PET findings to cognitive disability. Previous studies have shown that patients with cognitive or behavioral impairment have a worse prognosis.[2,31] How FDG-PET findings relate to cognitive testing requires additional study to further elucidate the mechanism by which frontotemporal hypometabolism is associated with worse prognosis as was found here.

Fourth, the accuracy of the classification analysis was based on a relatively small, but well-defined, control group of 20 sex- and age-matched participants. Enlargement of the control group may increase overall performance of the technique. Fifth, we have not defined a subset of patients and control cases as a training set on which subsequent cases could be evaluated, but we have used the leave-one-out technique to estimate future applicability in novel clinical samples. Whereas this is a necessary correction to establish robustness of classification accuracy, future studies in novel patient groups with clinical follow-up are needed to fully validate the current findings. Finally, the clinical diagnosis of ALS was used as the gold standard for diagnosis and compared with a single FDG-PET scan. We did not collect longitudinal FDG-PET data, perform FDG-PET around the time of symptom onset before a clinical diagnosis of ALS was made, nor assess the specificity of the FDG-PET changes to discriminate ALS from ALS mimics.

Conclusions

This study shows the promise on the individual patient level of an effective metabolic PET marker that has value in early and differential diagnosis in ALS and characterization of *C9orf72*-positive ALS. It also holds prognostic information, with frontotemporal metabolic decrease related to poorer survival.

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